

APPENDIX B: CLEAN COPY OF CLAIMS (UNOFFICIAL)

1. A method for producing disaggregated biologically active protein from a mixture comprising aggregated protein comprising the steps of:
 - (a) adjusting total protein concentration in the aggregate mixture to from about 0.01 mg/mL to about 500 mg/mL; then
 - (b) increasing the pressure on the aggregate mixture to from about 0.25 kbar to about 12 kbar for a time and temperature sufficient for disaggregation of the protein; then
 - (c) incubating the mixture under pressure in the range from about 0.25 kbar to about 3.3 kbar for a time from about 0.10 to about 12 hours; then
 - (d) reducing the pressure to atmospheric pressure, whereby protein in the mixture remains disaggregated and biological activity is retained.
2. The method of claim 1, wherein during the incubations step (c), the mixture further comprises an oxidizing agent and a reducing agent wherein the oxidizing agent is oxidized glutathione and the reducing agent is dithiothreitol.
3. The method of claim 1, wherein the pressure in the incubation step (c), is from about 0.5 kbar to about 3.3 kbar.
4. The method of claim 3, further comprising adding, prior to step (b), a chaotropic agent at a concentration of from about 0.1 to about 8 M.
5. The method of claim 4, wherein during the incubation step (c), the protein concentration is from about 1 to about 100 mg/mL.
6. The method of claim 4, wherein during the incubation step (c), the protein concentration is from about 1 to about 20 mg/mL.

7. The method of claim 4, wherein after step (c), the concentration of the chaotropic agent is decreased to less than about 0.1 M.
8. The method of claim 1, wherein, prior to step (a), the aggregated protein is treated with a reducing agent.
9. The method of claim 1, wherein the mixture of protein in step (a) comprises a detergent.
10. The method of claim 9, wherein the detergent is selected from the group consisting of sodium dodecyl sulfate, polyethoxysorbitan, deoxycholate, sodium octyl sulfate, sodium tetradecyl sulfate, polyoxyethylene ethers, sodium cholate, octylthioglucopyranoside, n-octylglucopyranoside, alkyltrimethylammonium bromides, alkyltrimethyl ammonium chlorides, and sodium bis (2-ethylhexyl) sulfosuccinate.
21. The method of claim 4, wherein the chaotropic agent is guanidine hydrochloride.
22. The method of claim 21, wherein guanidine hydrochloride is present at a concentration of from about 0.1 to about 1 M.